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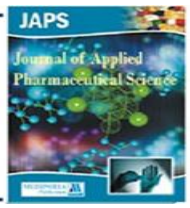

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Anti-inflammatory and antidiabetic activity of ethanolic extracts of *Sterculia villosa* barks on Albino Wistar rats

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ABSTRACT

Ethanolic extract of *Sterculia villosa* barks were studied for anti-inflammatory and antidiabetic activity. The acute oral toxicity showed that the ethanolic extract of *S. villosa* barks was safe until 4000mg/kg body weight and no macroscopical organ abnormalities were observed in acute oral models. The investigations on Albino (Wistar) rats at dosage of 100, 200 and 400 mg/kg of ethanolic extract of *Sterculia villosa* barks were made for aniti-inflammatory action by using carrageenan induced paw edema and cotton pellete granuloma technique. The results of the study suggested significant dose dependent activity of extracts as compared to control group for both acute and chronic inflammation. Ethanolic extract also showed significant antidiabetic activity at dose dependent manner compared to diabetic untreated group.

Keywords: *Sterculia villosa* barks, anti-inflammatory activity, antidibatic activity, carrageenan induced paw edema, cotton pellet granuloma, Alloxan.

INTRODUCTION

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells (Sosa *et al.*, 2002). It is a body defense reaction to eliminate or limit the spread of an injurious agent and is characterized by five cardinal signs, redness (*rubor*), swelling (*tumor*), heat (*calor*), pain (*dolor*) and loss of function (*function laesa*). The inflammatory process involves a cascade of events elicited by numerous stimuli that include infectious agents, ischemia, antigen-antibody interaction and thermal or physical injury (Broke *et al.*, 2006; Hunskaar *et al.*, 2006). Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of acute and chronic inflammation, pain and fever. But the greatest disadvantage in presently available synthetic drugs is that they cause gastrointestinal irritation and reappearance of symptoms after discontinuation. Therefore, there is a dire need for screening and development of novel, but better anti-inflammatory drugs and indigenous medicinal plants could be a logical source to find these.

Diabetes mellitus is a chronic disorder caused by partial or complete insulin deficiency, resulting in hyperglycemia leading to acute and chronic complications (Yadav *et al.*, 2008). The incidence of diabetes mellitus is on rise all over the world. Synthetic drugs are likely to give serious side effects in addition they are not suitable for intake during conditions like pregnancy (Larner *et al.*, 1985; Rao *et al.*, 1997; Valiathan *et al.*, 1998). Hence, search for a new drug with low cost, more potential, without adverse effects is being pursued in several laboratories all around the world.

Sterculis villosa Roxb, (Sterculiaceae), small to large, often spreading deciduous tree with grey or brown bark covered with corky nodules. It has large long-stalked deeply lobed leaves and yellow flowers. The plant is popularly known as 'Udal' or 'Udar' is abundantly available in the North Eastern Region of India. It is one of the fast-growing plant species. The plant is distributed in the tropical areas and widely distributed in Bangladesh, Srilanka and Southern China (Ghani *et al.*, 2003). Traditionally the plant is used in diuretic, cooling and aphrodisiac properties. The plant is used by Indians for traditional remedy of Inflamation (Namsa *et al.*, 2009). Some chemical constituents like, flavonoids, chrysoeriol, diosmetin-7-O-β-D-glucoside and chrysoeriol-7-O-β-D-glucoside were isolated from *S. villosa* (Seetharaman *et al.*, 1990). Pharmacological investigations of the plant have not been thoroughly explored. The plant also showed antioxidant activity (Kshirsagar *et al.*, 2009). In the traditional practice the bark of this plant has been used in diabetic and anti inflammatory agent. The claim that the anti-inflammatory activity of *S. villosa* barks is speculative and has not yet been documented. In the present study an attempt has been made to evaluate the anti-inflammatory efficacy of *S. villosa* barks in rat models.

MATERIALS AND METHODS

Plant material

Sterculia villosa Roxb, (Sterculiaceae) fresh barks collected from Rangamati, Bangladesh in sepetember 2011 were authenticated by the taxonomist Dr. Jasim Uddin, Associate Professor, Department of Botany, University of Dhaka and the identification number was documented as Accession no DUSH 6905 and Call no 01.

Preparation of the extract

The barks of *Sterculia villosa* Roxb, washed with distilled water to remove dirt and soil, then the barks were cut into small pieces and then dried. The dried materials were powdered and passed through a 10-mesh sieve. The coarsely powdered material was extracted with ethanol. The extracts were filtered, pooled and concentrated under reduced temperature on a rotary evaporator. The extract was stored in a refrigerator and used for the present study.

Test animals:

Albino (Wistar) rats 150-200 gm of either sex were used for the study. But, we selected only the male animals for antidiabetic activity since the females were reported, to be protected from lipid-Induced reduction in insulin action (Hevener *et al.*, 2002). The animals were kept in the standard polypropylene cages and provided with food and water ad libitum. The animals were housed under standard environmental conditions with controlled conditions of temperature (23 ± 2 ºC), humidity (50 ± 5 %) and 12 hour light-dark cycles. The animals were acclimatized for a period of 14 days prior to perform the experiments pharmacology. The experiments were performed according to the current guidelines for the care of the laboratory animals (Zimmerman et al., 1983).

Acute toxicity study

The Oral acute toxicity of ethanol extract of *Sterculia villosa* Roxb barks was determined in albino mice, maintained under standard conditions (Ecobichon *et al.*, 1997). The animals were fasted overnight prior to the experiment. Fixed dose (OCED Guideline no. 420) method of CPCSEA was adopted for toxicity studies (Prema et al., 2003). The tested extract was administrated orally. No mortality was observed at a dose of 4000mg/kg.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated by two models carrageenan induced paw edema and cotton pellet granuloma.

Carrageenan-induced paw edema

The five groups of rats, six in each group was included in this study. Inflammation was induced by injecting 0.1ml of 1% w/v carrageenan sodium salt subcutaneously in the sub-plantar region of the rat right hind paw (Winter *et al.*, 1962).

The *S. villosa* root extract (100, 200, 400mg/kg) or Diclofenac sodium (5 mg/kg) was administered orally 1 hour before carrageenan injection while control group received only saline at the doses of 10ml/kg body weight. The hind paw volume was measured plethysmometrically before and after the carrageenan injection, at hourly intervals for 3hr.

$$\% \text{ of inhibition of edema} = (V_c - V_t)/V_c \times 100$$

Where, V_t = mean paw volume of test group & V_c = mean paw volume of control group.

Cotton pellet granuloma

The five groups of rats, six in each group was included in this study. For cotton pellet granuloma, a 50 mg sterilized cotton pellet was implanted subcutaneously on the back of neck in rats under ether anesthesia.

Animals in the control group received only the vehicle at the dose of 10ml/kg. Animals in treated group received the extract at the doses of 100, 200 and 400 mg/kg body weight once daily for 14 consecutive days. Diclofenac sodium (5mg/kg) was given as reference drug in a fourth group. On the 14th day the animals were sacrificed with ether, the pellets granulomas were removed, fixed from extraction tissue, dried overnight at 55±0.5ºC and weighed (Winter et al., 1957).

Induction of Diabetes

Hyperglycemia was induced in overnight fasted Albino Waster rats by a single intraperitonial injection of freshly prepared Alloxan Monohydrate in sterile saline at a dose of 120mg/kg body weight (Ju *et al.,* 2008). After 5 days of Alloxan injection, the diabetic rats (glucose level >250 mg/dl) were selected and grouped for the study.

Evaluation of antidiabetic activity

Diabetes was induced in Albino Wistar rats by intraperitonial administration of ice cold aqueous Alloxan Monohydrate (Rao *et al.,* 1999). The fasting blood sugar levels of each of the rats were checked every day with an autoanlyzer (Glucometer, Bioland G-423 S) glucose kit. After 8 days, animals with fasting blood sugar levels of 250 mg/dl and above were considered to be diabetic and were used for the study. The selected rats were divided into five groups of six rats each like Group I (Diabetic untreated rats received Tween-80 solution), Group II, III & IV (Diabetic rats treated with ethanol extract of *S. villosa* root extract at the dose of 250, 500 & 1000mg/kg) and Group V (diabetic rats treated with standard reference drug Glibenclamide 5mg tablet of Daonil from Sanofi-Aventis at a dose of 5mg/kg).

After the administration of drug and extracts blood glucose levels of the rats were measured at hourly intervals of 0, 1, 2 and 3 hours. Blood samples were then collected by tail snip and the blood glucose measured with an autoanalyzer glucose kit (Glucometer, Bioland G-423 S). At the end of the experiment, percentage reduction of the glucose levels of the rats at the 3rd hour was calculated using the following formula:

Percentage Reduction = [(BGL at 0 hr – BGL at 3rd hr)/BGL at 3rd hr] X 100%

BGL = Blood Glucose Level

Statistical Analysis

The result were expressed as Mean ± SEM. Statistical Analysis was performed with one way analysis of variance (ANOVA) followed by student's t' test. P values less than 0.05 were considered to be statistically significant, when compared with control.

Results and Discussion

The present study establishes the anti-inflammatory activity of *S. villosa* barks at different doses employed for screening of different phases of inflammatory process. The development of carrageenan induced edema is believed to be biphasic of which the first phase is mediated by release of histamine, seritonin and kinins in the first hour after injection of carrageenan and the second phase is related to release of prostaglandin like substances in 2-3 hours (Brooks *et al.,* 1991). Results of the present study are suggesting that the root extract predominantly inhibits the release of prostaglandin like substances from phlogenic stimuli. In addition flavonoid possesses anti-inflammatory activity and some of them also act as phospholipase inhibitors, such inhibitors are able to decrease inflammatory response to carrageenan in the rats (Ferrandiz *et al.,* 1991; Fowzy *et al.,* 1988; Aitchdrfoun *et al.,* 1996). The result of anti-inflammatory effect of *S. villosa* barks on carrageenan induced paw has been shown in Table 1.

The results of current study for anti-inflammatory activity of *S. villosa* barks against the cotton pellet granuloma technique (Table 2) established the anti-inflammatory activity of the root extract at different doses. The extract showed significant ($p<0.05$) anti-inflammatory effect in dose dependent manner. The repairing phase of inflammation is initiated as a proliferation of fibroblasts and a multiplication of small blood vessels. Proliferating cells penetrate the exudates, producing a highly vascularized reddened mass known as granulation tissue (Miyake et al., 1993). Significant reduction of the cotton pellete induced granuloma in rats by *S. villosa* barks suggested that the extract has the activity in the proliferative phase of inflammatory process.

Antidiabetic Activity of Diabetic Induced Rats

Elevation of blood glucose level by Alloxan, a β-cytotoxin, due to reduced synthesis and release of insulin as a result of massive destruction of β-cells of the islets of Langerhans confirmed the induction of diabetes in alloxan-induced experimental rats (Lazarow *et al.,* 1964). In the present study, hyperglycemia produced by alloxan monohydrate was significantly lowered (Table 3) by administration of ethanol extract of *S. villosa* barks in a dose of 250, 500 and 1000 mg/kg body weight after 3 hour of treatment.

Table 3 shows the blood glucose levels of diabetic control, ethanol extract of *S. villosa* barks and glibenclamide-treated rats. In diabetic control rats, the increase in blood glucose concentration was observed after 1 h. The blood glucose concentration remained high over the next hour. Ethanol extract of *S. villosa* barks and glibenclamide treated rats showed significant decrease ($p<.05$) in blood glucose concentration at hourly intervals when compared with diabetic control rats. The antidiabetic studies of the ethanol extract of *S. villosa* barks on alloxan induced diabetic rats showed highly significant antidiabetic effect with minimal toxicity.

Table. 1: Effect of ethanolic extract of *S. villosa* barks on carrageenan induced paw edema in rats.

Treatment	Dose (mg/kg)	Paw volume ml (Mean ± SEM)				% inhibition after 3rd hour
		0 hrs	1 hrs	2 hrs	3 hrs	
Control		0.75± 0.04	1.12± 0.04	1.19± 0.04	1.23± 0.04	-
Diclofenac	5	0.73± 0.02	0.91± 0.02	0.83± 0.04	0.80± 0.04	34.95
S. villosa barks	100	0.71± 0.03	1.05± 0.03	1.00± 0.02	0.97± 0.02	21.13*
S. villosa barks	200	0.72± 0.03	1.01± 0.01	0.98± 0.02	0.94± 0.02	23.57*
S. villosa barks	400	0.72± 0.01	0.97± 0.04	0.93± 0.03	0.90± 0.02	26.83*

Values are Mean ± SEM (n = 6). *p<.05 as compared to carrageenan control.

Table. 2: Effect of ethanolic extract of *S. villosa* barks on cotton pellet induced granuloma in rats.

Treatment	Dose (mg/kg/day)	Mean weight of Granuloma (mg) (Mean ± SEM)
Control		101.17 ± 1.35
Diclofenac	5	62.75 ± 1.48
S. villosa barks	100	92.23 ± 1.33 **
S. villosa barks	200	80.33 ± 1.28 **
S. villosa barks	400	71.24 ± 0.86 **

Values are Mean ± SEM (n= 6); **p<.05 as compared to control.

Table. 3: Effect of ethanol extract of *S. villosa* barks (EESVB) on the fasting blood glucose levels of Alloxan induced diabetic rats.

Group	Treatment	Dose (mg/kg body weight)	Fasting Blood Glucose Level (mg/ml) (Mean±SEM)				% reduction at the 3rd hour
			0 hr	1 hr	2 hr	3 hr	
I	Diabetic untreated		384.6±6.4	388.1±5.4	391.2±4.6	387.6±5.1	-
II	Diabetic treated with EESVB	250	385.1±5.9	342.6±5.1	325.3±5.1	288.9±5.5	24.98
III	Diabetic treated with EESVB	500	395.5±6.8	333.1±3.6	307.9±3.6	263.1±4.7	33.47 ***
IV	Diabetic treated with EESVB	1000	377.3±5.8	311.3±4.4.2	287.8±4.8	235.7±4.1	37.53 ***
V	Diabetic treated with Glibenclamide	5	368.1±4.9	181.7±2.3	108.3±1.7	95.8±1.2	73.97 ***

Values are Mean ± SEM (n= 6); ***p<.05 as compared to control.

CONCLUSION

Anti-inflammatory activities of many plants have been attributed to their high sterol/triterpenoid saponins (Gupta *et al.*, 1969). Though at this stage it is not possible to identify the exact phytochemical constituents responsible for anti-inflammatory activities of *S. villosa* barks, it may be assumed that the effects could be due chemicals present in the ethanol extract. The result of present study indicates that ethanol extract of *S. villosa* barks possess significant anti-inflammatory activity on both acute and chronic inflammation. Further detailed investigation is underway to determine the exact phytoconstituents, which are responsible for the anti-inflammatory activity.

Alloxan causes massive reduction in insulin release, through the destruction of ß-cells of the islets of Langerhans. In our study, we have observed a significant increase in the plasma insulin level when alloxan diabetic rats were treated with ethanol extract of *S. villosa* barks. This could be due to potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing ß-cells of islets of Langerhans or its release from bound insulin. The significant and consistent antidiabetic effect of ethanol extract of *S. villosa* barks in alloxan diabetic rats may also be due to enhanced glucose utilization by peripheral tissues. Further studies will be done to determine the exact chemical(s) & mechanism of action responsible for antidiabetic activity.

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